WEST Search History

DATE: Friday, July 18, 2003

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,PGPB,DWPI; PLUR=YES; OP=ADJ			
L10	l8 not l5	157	L10
L9	l8 not l3	117	L9
L8	l6 and L7	168	L8
L7	rgd	2761	L7
L6	angiostatin or endostatin	1415	L6
L5	l3 same L4	13	L5
L4	chimeric or complex$2 or conjugat$3	811575	L4
L3	l1 same L2	168	L3
L2	angiogene$	12780	L2
L1	rgd	2761	L1

END OF SEARCH HISTORY

WEST Search History

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Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,PGPB,DWPI; PLUR=YES; OP=ADJ			
L5	l3 same L4	13	L5
L4	chimeric or complex$2 or conjugat$3	811575	L4
L3	l1 same L2	168	L3
L2	angiogene$	12780	L2
L1	rgd	2761	L1

END OF SEARCH HISTORY

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FILE COVERS 1969 TO DATE.
CAS REGISTRY NUMBERS AND CHEMICAL NAMES (CNs) PRESENT
FROM JANUARY 1969 TO DATE.

RECORDS LAST ADDED: 16 July 2003 (20030716/ED)

=> s rgd and angiogene?
2963 RGD
20072 ANGIOGENE?
L1 105 RGD AND ANGIOGENE?

=> s complex? or conjugat? or chimeric
520632 COMPLEX?
76957 CONJUGAT?
20958 CHIMERIC
L2 609823 COMPLEX? OR CONJUGAT? OR CHIMERIC

=> s l1 and l2
L3 18 L1 AND L2

=> d 1-18 bib,ab

L3 ANSWER 1 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2003:332147 BIOSIS
DN PREV200300332147
TI A recombinant **chimeric** epidermal growth factor-like module with high binding affinity for integrins.
AU Vella, Fanny; Thielens, Nicole M.; Bersch, Beate; Arlaud, Gerard J.; Frachet, Philippe (1)
CS (1) Laboratoire d'Enzymologie Moleculaire, Institut de Biologie Structurale Jean-Pierre Ebel, 41 Rue Jules Horowitz, Grenoble Cedex 1, 38027, France: philippe.frachet@ibs.fr France
SO Journal of Biological Chemistry, (May 30 2003) Vol. 278, No. 22, pp. 19834-19843. print.
ISSN: 0021-9258.
DT Article
LA English
AB Integrins are cell surface receptors involved in numerous pathological processes such as metastasis invasion and abnormal **angiogenesis**. To target these receptors, the epidermal growth factor (EGF)-like domain of human complement protease C1r was used as a natural scaffold to design **chimeric** modules containing the **RGD** motif. Here we report a high yield bacterial expression system and its application to the production of two such modules, EGF-**RGD** and V2, the latter variant mimicking the **RGD**-containing domain of disintegrins. These modules were characterized chemically, and their biological activity was investigated by cellular assays using various Chinese hamster ovary cell lines expressing beta1 and beta3 integrins and by surface plasmon resonance spectroscopy. Remarkably, the modifications leading to the V2 variant had differential effects on the interaction with beta3 and beta1 integrins. The disintegrin-like V2 module exhibited enhanced binding affinities compared with EGF-**RGD**, with KD values of 7.2 nM for alpha5beta1 (a 4-fold decrease) and 3.5 nM for alphavbeta3 (a 1.5-fold

decrease), comparable with the values determined for natural integrin ligands. Analysis by NMR spectroscopy also revealed a differential dynamic behavior of the **RGD** motif in the EGF-**RGD** and V2 variants, providing insights into the structural basis of their relative binding efficiency. These novel **RGD**-containing EGF modules open the way to the design of improved variants with selective affinity for particular integrins and their use as carriers for other biologically active modules.

L3 ANSWER 2 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2003:246012 BIOSIS
DN PREV200300246012
TI Photocrosslinked hyaluronic acid hydrogels: Natural, biodegradable tissue engineering scaffolds.
AU Leach, Jennie Baier; Bivens, Kathryn A.; Patrick, Charles W., Jr.; Schmidt, Christine E. (1)
CS (1) Department of Biomedical Engineering, University of Texas at Austin, 26th and Speedway, MC CO400, Austin, TX, 78712, USA: schmidt@che.utexas.edu USA
SO Biotechnology and Bioengineering, (June 5 2003) Vol. 82, No. 5, pp. 578-589. print.
ISSN: 0006-3592.
DT Article
LA English
AB Ideally, rationally designed tissue engineering scaffolds promote natural wound healing and regeneration. Therefore, we sought to synthesize a biomimetic hydrogel specifically designed to promote tissue repair and chose hyaluronic acid (HA; also called hyaluronan) as our initial material. Hyaluronic acid is a naturally occurring polymer associated with various cellular processes involved in wound healing, such as **angiogenesis**. Hyaluronic acid also presents unique advantages: it is easy to produce and modify, hydrophilic and nonadhesive, and naturally biodegradable. We prepared a range of glycidyl methacrylate-HA (GMHA) **conjugates**, which were subsequently photopolymerized to form crosslinked GMHA hydrogels. A range of hydrogel degradation rates was achieved as well as a corresponding, modest range of material properties (e.g., swelling, mesh size). Increased amounts of **conjugated** methacrylate groups corresponded with increased crosslink densities and decreased degradation rates and yet had an insignificant effect on human aortic endothelial cell cytocompatibility and proliferation. Rat subcutaneous implants of the GMHA hydrogels showed good biocompatibility, little inflammatory response, and similar levels of vascularization at the implant edge compared with those of fibrin positive controls. Therefore, these novel GMHA hydrogels are suitable for modification with adhesive peptide sequences (e.g., **RGD**) and use in a variety of wound-healing applications.

L3 ANSWER 3 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2003:184997 BIOSIS
DN PREV200300184997
TI In vitro cell studies of technetium-99m labeled **RGD**-HYNIC peptide, a comparison of tricine and EDDA as co-ligands.
AU Su, Zi-Fen; He, Jiang; Rusckowski, Mary; Hnatowich, Donald J. (1)
CS (1) Division of Nuclear Medicine, Department of Radiology, Medical School, University of Massachusetts, Worcester, MA, 01655, USA: donald.hnatowich@umassmed.edu USA
SO Nuclear Medicine and Biology, (February 2003) 2003) Vol. 30, No. 2, pp. 141-149. print.
ISSN: 0969-8051.
DT Article
LA English
AB The level of alphavbeta3 integrins on endothelial cells is elevated in **angiogenesis**. The high binding specificity to alphavbeta3 integrins of peptides containing Arg-Gly-Asp (**RGD**) residues

suggests that the radiolabeled **RGD** peptides may be useful as tumor specific imaging agents. In this research, cyclised peptides containing Arg-Gly-Asp (**RGD**) and Arg-Gly-Glu (RGE, as control) residues were **conjugated** with HYNIC and labeled with 99mTc. Objective: The goal was to evaluate the influence of co-ligand, either tricine or ethylenediamine-N,N'-diacetic acid (EDDA) on protein and integrin binding and on cellular uptake in culture. Methods: The n-octanol/water partition coefficient, binding to bovine serum albumin (BSA) and human umbilical vein endothelial (HUVE) cells, and cell lysate distributions of the radiolabeled peptides were evaluated. Results: The co-ligands had a significant effect on the labeling efficiency of the HYNIC **conjugates** and on certain properties of the 99mTc **complexes**. The labeling efficiency with tricine was 10 fold higher and BSA binding was over 8 fold greater compared to EDDA. Both **RGD** labels showed higher (6 to 28 fold) binding to HUVE cells than that of the RGE labels, indicating binding specificity. After cell-lysis, only a small percentage of the total **RGD** label that accumulated in the cells was found bound to cellular proteins (9% of **RGD**/tricine and 5% of **RGD**/EDDA), implying that over 90% of the radiolabeled peptides were internalized for both radiolabeled RGDs. The number of the **RGD** molecules bound to proteins was estimated to be approximately three per cell, suggesting that only a small number of alphavbeta3 integrin proteins are expressed on the cells. Conclusions: Apart from the differences in radiolabeling, the only important effect of substituting EDDA for tricine as co-ligand on the HYNIC-peptides was the lower degree of serum protein binding. In spite of the lower serum protein binding potential, in vivo tumor accumulation of the **RGD**/EDDA may not be improved compared to **RGD**/tricine since quantitation of the cell binding results suggests that the number of alphavbeta3 integrin proteins per cell might be limited.

L3 ANSWER 4 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2003:17784 BIOSIS
DN PREV200300017784
TI Targeting of **RGD**-modified proteins to tumor vasculature: A pharmacokinetic and cellular distribution study.
AU Schraa, Astrid J. (1); Kok, Robbert J.; Moorlag, Henk E.; Bos, Erwin J.; Proost, Johannes H.; Meijer, Dirk K. F.; de Leij, Lou F. M. H.; Molema, Grietje
CS (1) Dept. of Pathology and Laboratory Medicine, Medical Biology Section, Tumor Immunology Laboratory, 9700 RB, PO Box 30.001, Groningen, Netherlands: a.j.schraa@med.rug.nl Netherlands
SO International Journal of Cancer, (10 December 2002) Vol. 102, No. 5, pp. 469-475. print.
ISSN: 0020-7136.
DT Article
LA English
AB **Angiogenesis**-associated integrin alphavbeta3 represents an attractive target for therapeutic intervention because it becomes highly upregulated on angiogenic endothelium and plays an important role in the survival of endothelial cells. Cyclic **RGD** peptides were prior shown to have a high affinity for alphavbeta3 and can induce apoptosis of endothelial cells. In our laboratory, monocyclic **RGD** peptides (cRGDfK) were chemically coupled to a protein backbone. Previous results demonstrated that the resulting RGDpep-HuMab **conjugate** bound with increased avidity to alphavbeta3/alphavbeta5 on endothelial cells. In our present study, RGDpep-HuMab was injected intravenously and intraperitoneally in B16.F10 tumor-bearing mice to determine its pharmacokinetics and organ distribution. In the tumor, the RGDpep-HuMab **conjugate** specifically localized at the endothelium as was demonstrated by immunohistochemistry. The control RADpep-HuMab **conjugate** was not detected in the tumor. Besides tumor localization RGDpep-HuMab was found in liver and spleen associated with macrophages. This uptake by macrophages is probably responsible for the

more rapid clearance of RGDpep-HuMab from the circulation than HuMab and RADpep-HuMab. The half-life of RGDpep-HuMab (90 min) was still considerably longer than that of free **RGD** peptides (<10 min). This prolonged circulation time may be favorable for drug targeting strategies because the target cells are exposed to the **conjugate** for a longer time period. Taken together these results indicate that **RGD**-modified proteins are suitable carriers to deliver therapeutic agents into tumor or inflammation induced angiogenic endothelial cells.

L3 ANSWER 5 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2002:602715 BIOSIS
DN PREV200200602715
TI Tumor targeting with radiolabeled alphavbeta3 integrin binding peptides in a nude mouse model.
AU Janssen, Marcel L. (1); Oyen, Wim J.; Dijkgraaf, Ingrid; Massuger, Leon F.; Frielink, Cathelijne; Edwards, D. Scott; Rajopadhye, Milind; Boonstra, Henk; Corstens, Frans H.; Boerman, Otto C. (1)
CS (1) Department of Nuclear Medicine, UMC Nijmegen, 6500 HB, P. O. Box 9101, Nijmegen Netherlands
SO Cancer Research, (November 1, 2002) Vol. 62, No. 21, pp. 6146-6151. http://cancerres.aacrjournals.org/. print.
ISSN: 0008-5472.
DT Article
LA English
AB The alphavbeta3 integrin is expressed on proliferating endothelial cells such as those present in growing tumors, as well as on tumor cells of various origin. Tumor-induced **angiogenesis** can be blocked in vivo by antagonizing the alphavbeta3 integrin with small peptides containing the Arg-Gly-Asp (**RGD**) amino acid sequence. This tripeptidic sequence, naturally present in extracellular matrix proteins, is the primary binding site of the alphavbeta3 integrin. Because of selective expression of alphavbeta3 integrin in tumors, radiolabeled **RGD** peptides are attractive candidates for alphavbeta3 integrin targeting in tumors. We studied the in vivo behavior of the radiolabeled dimeric **RGD** peptide E-(c(RGDfK))2 in the NIH:OVCAR-3 s.c. ovarian carcinoma xenograft model in BALB/c nude mice. **Conjugation** of the 1,4,7,10-tetraazadodecane-N,N',N",N'''-tetraacetic acid (DOTA) and hydrazinonicotinamide (HYNIC) chelators enabled efficient radiolabeling with 111In/90Y and 99mTc, respectively. The radiolabeled peptide was rapidly excreted renally. Uptake in nontarget organs such as liver and spleen was considerable. Tumor uptake peaked at 7.5% injected dose (ID)/g (111In-DOTA-E-(c(RGDfK))2) or 6.0%ID/g (99mTc-HYNIC-E-(c(RGDfK))2) at 2 and 1 h postinjection, respectively. Integrin alphavbeta3 receptor binding specificity was demonstrated by reduced tumor uptake after injection of the scrambled control peptide 111In-DOTA-E-(c(RD-KfD))2 (0.28%ID/g at 2 h p.i.) and after coinjection of excess nonradioactive 115In-DOTA-E-(c(RGDfK))2 (0.22%ID/g at 2 h p.i.). A single injection of 90Y-DOTA-E-(c(RGDfK))2 at the maximum-tolerated dose (37 MBq) in mice with small s.c. tumors caused a significant growth delay as compared with mice treated with 37 MBq 90Y-labeled scrambled peptide or untreated mice (median survival of 54 versus 33.5 versus 19 days, respectively). In conclusion, the radiolabeled **RGD** peptides 111In-DOTA-E-(c(RGDfK))2 and 99mTc-HYNIC-E-(c(RGDfK))2 demonstrated high and specific tumor uptake in a human tumor xenograft. Injection of 90Y-DOTA-E-(c(RGDfK))2 induced a significant delay in tumor growth. Potentially, these peptides can be used for peptide receptor radionuclide imaging as well as therapy.

L3 ANSWER 6 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2002:356763 BIOSIS
DN PREV200200356763
TI In vitro and in vivo evaluation of a technetium-99m-labeled cyclic **RGD** peptide as a specific marker of alphavbeta3 integrin for tumor imaging.

AU Su, Zi-Fen; Liu, Guozheng; Gupta, Suresh; Zhu, Zhihong; Rusckowski, Mary; Hnatowich, Donald J. (1)
CS (1) Division of Nuclear Medicine, Department of Radiology, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA, 01655: donald.hnatowich@umassmed.edu USA
SO Bioconjugate Chemistry, (May June, 2002) Vol. 13, No. 3, pp. 561-570. http://pubs.acs.org/journals/bcches/. print.
ISSN: 1043-1802.
DT Article
LA English
AB Three amino acids residues, Arg-Gly-Asp (**RGD**), in vitronectin and fibronectin show affinity for alphavbeta3 integrins expressed in vascular endothelial cells. That tumor growth can upregulate the expression of these integrins on tumor cells for invasion and metastasis and in tissue neovasculature suggests the potential of developing radiolabeled **RGD** peptides as antagonists of alphavbeta3 integrins for broad spectrum tumor specific imaging. The polypeptide **RGD**-4C, which contains four cysteine residues for cyclization, has shown preferential localization on integrins at sites of tumor **angiogenesis**. Both **RGD**-4C and RGE (Arg-Gly-Glu)-4C (as control) were purchased and **conjugated** with 6-hydrazinopyridine-3-carboxylic acid (HYNIC) for 99mTc radiolabeling. After purification of the **conjugated** peptides by a C18 Sep-Pak cartridge with 20% methanol, both peptides were radiolabeled using tricine. For cell binding studies, both 99mTc peptides were further purified by SE HPLC. High specific radioactivity of labeled cyclized **RGD**/E (cyclized **RGD**/E will be simplified as **RGD**/E through out the text) of about 20 Ci/mumol was achieved. Both 99mTc **complexes** were stable in the labeling solution for over 24 h at room temperature. In the human umbilical vein endothelial (HUVE) cell studies, the binding at 1 h of radiolabeled **RGD**/E was determined at 4 degreeC and at concentrations in the picomolar to nanomolar range. Under these conditions, cell accumulation of 99mTc in the case of **RGD** was as much as 16 times greater than the control RGE. As a check on specificity, 7 nM of native cyclized **RGD** blocked 50% of the binding of 99mTc-labeled **RGD** to cells. The binding percentage of 99mTc-labeled **RGD** to purified alphavbeta3 integrin protein, as determined by SE HPLC, increased with the concentration of the integrin while 99mTc-labeled RGE showed no binding. The association constant for 99mTc-**RGD** was modest at 7 X 106 M-1. In both human renal adenocarcinoma (ACHN) and human colon cancer cell line (LS174T) nude mouse tumor models, the accumulation of 99mTc-labeled **RGD**/E exhibited no statistical difference. In conclusion, possibly because of limited numbers of alphavbeta3 integrin receptors per tumor cell and low binding affinity, radiolabeled **RGD** peptides may have limitations as tumor imaging agents.

L3 ANSWER 7 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2002:190465 BIOSIS
DN PREV200200190465
TI Preparation and functional evaluation of **RGD**-modified proteins as alphavbeta3 integrin directed therapeutics.
AU Kok, Robbert J. (1); Schraa, Astrid J.; Bos, Erwin J.; Moorlag, Henk E.; Asgeirsdottir, Sigridur A.; Everts, Maaike; Meijer, Dirk K. F.; Molema, Grietje
CS (1) Department of Pharmacokinetics and Drug Delivery, University Centre for Pharmacy, Groningen University Institute for Drug Exploration (GUIDE), A. Deusinglaan 1, 9713 AV, Groningen: r.j.kok@farm.rug.nl Netherlands
SO Bioconjugate Chemistry, (January February, 2002) Vol. 13, No. 1, pp. 128-135. http://pubs.acs.org/journals/bcches/. print.
ISSN: 1043-1802.
DT Article
LA English
AB Tumor blood vessels can be selectively targeted by **RGD**-peptides

that bind to alphavbeta3 integrin on angiogenic endothelial cells. By inhibiting the binding of these integrins to its natural ligands, **RGD**-peptides can serve as antiangiogenic therapeutics. We have prepared multivalent derivatives of the cyclic **RGD**-peptide c(RGDfK) by covalent attachment of the peptide to side chain amino groups of a protein. These RGDpep-protein **conjugates** inhibited alphavbeta3-mediated endothelial cell adhesion in vitro, while **conjugates** prepared with a control RAD-peptide showed no activity. Radiobinding and displacement studies with endothelial cells demonstrated an increased affinity of the RGDpep-protein **conjugates** compared to the free peptide, with IC50 values ranging from 23 to 0.6 nM, depending on the amount of coupled RGDpep per protein. Compared to the parental **RGD**-peptide and the related **RGD**-peptide ligand c(RGDfV), the RGDpep-protein **conjugates** showed a considerable increase in affinity (IC50 parent RGDpep: 818 nM; IC50 c(RGDfV): 158 nM). We conclude that the **conjugation** of **RGD**-peptides to a protein, resulting in products that can bind multivalently, is a powerful approach to increase the affinity of peptide ligands for alphavbeta3/alphavbeta5 integrins.

L3 ANSWER 8 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2001:392614 BIOSIS
DN PREV200100392614
TI Improved pharmacokinetics of (18F)**RGD**-peptides by serine-**conjugation**.
AU Haubner, R. (1); Wester, H. J. (1); Weber, W. A. (1); Linke, W. (1); Bodenstein, C. (1); Kessler, H.; Schwaiger, M. (1)
CS (1) Department of Nuclear Medicine, Technische Universitaet Muenchen, D-81675, Muenchen Germany
SO Journal of Labelled Compounds and Radiopharmaceuticals, (May, 2001) Vol. 44, No. Supplement 1, pp. S157-S159. print.
Meeting Info.: Fourteenth International Symposium on Radiopharmaceutical Chemistry Interlaken, Switzerland June 10-15, 2001
ISSN: 0362-4803.
DT Conference
LA English
SL English

L3 ANSWER 9 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2001:123999 BIOSIS
DN PREV200100123999
TI Aberrant fibrin formation and cross-linking of fibrinogenNieuwegein, a variant with a shortened Aalpha-chain, alters endothelial capillary tube formation.
AU Collen, Annemie; Maas, Annemarie; Kooistra, Teake; Lupu, Florea; Grimbergen, Jos; Haas, Fred J. L. M.; Biesma, Douwe H.; Koolwijk, Pieter; Koopman, Jaap; van Hinsbergh, Victor W. M. (1)
CS (1) Gaubius Laboratory, TNO-PG, Zemikedreef 9, 2333 CK, Leiden: vwm.vanhinsbergh@pg.tno.nl Netherlands
SO Blood, (February 15, 2001) Vol. 97, No. 4, pp. 973-980. print.
ISSN: 0006-4971.
DT Article
LA English
SL English
AB A congenital dysfibrinogenemia, fibrinogenNieuwegein, was discovered in a young man without any thromboembolic complications or bleeding. A homozygous insertion of a single nucleotide (C) in codon Aalpha 453 (Pro) introduced a stop codon at position 454, which resulted in the deletion of the carboxyl-terminal segment Aalpha 454-610. The ensuing unpaired cysteine at Aalpha 442 generated fibrinogen-albumin **complexes** of different molecular weights. The molecular abnormalities of fibrinogenNieuwegein led to a delayed clotting and a fibrin network with a low turbidity. Electron microscopy confirmed that thin fibrin bundles were organized in a fine network. The use of fibrinogenNieuwegein-derived

fibrin (fibrinNieuwegein) in an in vitro **angiogenesis** model resulted in a strong reduction of tube formation. The ingrowth of human microvascular endothelial cells (hMVEC) was independent of alphavbeta3, indicating that the reduced ingrowth is not due to the absence of the **RGD**-adhesion site at position Aalpha 572-574. Rather, the altered structure of fibrinNieuwegein is the cause, since partial normalization of the fibrin network by lowering the pH during polymerization resulted in an increased tube formation. Whereas factor XIIIa further decreased the ingrowth of hMVEC in fibrinNieuwegein, tissue transglutaminase (TG), which is released in areas of vessel injury, did not. This is in line with the absence of the cross-linking site for TG in the alpha-chains of fibrinogenNieuwegein. In conclusion, this newly discovered congenital dysfibrinogenemia has a delayed clotting time and leads to the formation of an altered fibrin structure, which could not be cross-linked by TG and which is less supportive for ingrowth of endothelial cells.

L3 ANSWER 10 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2000:264402 BIOSIS
DN PREV200000264402
TI Lidamycin and its **RGD**-containing peptide **conjugate** inhibit **angiogenesis** and metastasis.
AU Zhen, Yong-Su (1); Lin, M.; Zhen, H. Y.; Wang, X. H.
CS (1) Inst of Medicinal Bio, Chinese Acad of Med Sci, Beijing China
SO Proceedings of the American Association for Cancer Research Annual Meeting, (March, 2000) No. 41, pp. 645. print..
Meeting Info.: 91st Annual Meeting of the American Association for Cancer Research. San Francisco, California, USA April 01-05, 2000
ISSN: 0197-016X.
DT Conference
LA English
SL English

L3 ANSWER 11 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2000:24068 BIOSIS
DN PREV200000024068
TI Tensional forces in fibrillar extracellular matrices control directional capillary sprouting.
AU Korff, Thomas; Augustin, Hellmut G. (1)
CS (1) Cell Biology Laboratory, Department of Gynecology and Obstetrics, University of Goettingen Medical School, 37075, Goettingen Germany
SO Journal of Cell Science, (Oct., 1999) Vol. 112, No. 19, pp. 3249-3258.
ISSN: 0021-9533.
DT Article
LA English
SL English
AB During **angiogenesis**, anastomosing capillary sprouts align to form **complex** three-dimensional networks of new blood vessels. Using an endothelial cell spheroid model that was developed to study endothelial cell differentiation processes, we have devised a novel collagen gel-based three-dimensional in vitro **angiogenesis** assay. In this assay, cell number-defined, gel-embedded endothelial cell spheroids act as a cellular delivery device, which serves as a focal starting point for the sprouting of lumenized capillary-like structures that can be induced to form **complex** anastomosing networks. Formation of capillary anastomoses is associated with tensional remodeling of the collagen matrix and directional sprouting of outgrowing capillaries towards each other. To analyze whether directional sprouting is dependent on cytokine gradients or on endothelial cell-derived tractional forces transduced through the extracellular matrix, we designed a matrix tension generator that enables the application of defined tensional forces on the extracellular matrix. Using this matrix tension generator, causal evidence is presented that tensional forces on a fibrillar extracellular matrix such as type I collagen, but not fibrin, are sufficient to guide directional outgrowth of endothelial cells. **RGD** peptides but not

control RAD peptides disrupted the integrity of sprouting capillary-like structures and induced detachment of outgrowing endothelial cells cultured on top of collagen gels, but did not inhibit primary outgrowth of endothelial cells. The data establish the endothelial cell spheroid-based three-dimensional **angiogenesis** technique as a standardized, highly reproducible quantitative assay for in vitro **angiogenesis** studies and demonstrate that integrin-dependent matrix tensional forces control directional capillary sprouting and network formation.

L3 ANSWER 12 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1999:310032 BIOSIS
DN PREV199900310032
TI Vitronectin.
AU Schvartz, Iris; Seger, Dalia; Shaltiel, Shmuel (1)
CS (1) Department of Biological Regulation, Weizmann Institute of Science, IL-76100, Rehovot Israel
SO International Journal of Biochemistry & Cell Biology, (May, 1999) Vol. 31, No. 5, pp. 539-544.
ISSN: 1357-2725.
DT Article
LA English
SL English
AB Vitronectin is a multifunctional glycoprotein present in blood and in the extracellular matrix. It binds glycosaminoglycans, collagen, plasminogen and the urokinase-receptor, and also stabilizes the inhibitory conformation of plasminogen activation inhibitor-1. By its localization in the extracellular matrix and its binding to plasminogen activation inhibitor-1, vitronectin can potentially regulate the proteolytic degradation of this matrix. In addition, vitronectin binds to complement, to heparin and to thrombin-antithrombin III **complexes**, implicating its participation in the immune response and in the regulation of clot formation. The biological functions of vitronectin can be modulated by proteolytic enzymes, and by exo- and ecto-protein kinases present in blood. Vitronectin contains an **RGD** sequence, through which it binds to the integrin receptor alphavbeta3, and is involved in the cell attachment, spreading and migration. Antibodies against alphavbeta3 or synthetic peptides containing an **RGD** sequence are now being tested as therapeutic agents in the treatment of human cancers, bone diseases (e.g. osteoporosis) and in pathological disorders which involve **angiogenesis**.

L3 ANSWER 13 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1999:244761 BIOSIS
DN PREV199900244761
TI Del1 induces integrin signaling and **angiogenesis** by ligation of alphaVbeta3.
AU Penta, Kalyani; Varner, Judith A.; Liaw, Lucy; Hidai, Chiaki; Schatzman, Randy; Quertermous, Thomas (1)
CS (1) Div. of Cardiology, Stanford University Medical School, 300 Pasteur Dr., Falk Bldg., Stanford, CA, 94305-5406 USA
SO Journal of Biological Chemistry, (April 16, 1999) Vol. 274, No. 16, pp. 11101-11109.
ISSN: 0021-9258.
DT Article
LA English
SL English
AB Del1 is a novel extracellular matrix protein encoding three Notch-like epidermal growth factor repeats, an **RGD** motif, and two discoidin domains. Del1 is expressed in an endothelial cell-restricted pattern during early development. In studies reported here, recombinant baculovirus Del1 protein was shown to promote alphavbeta3-dependent endothelial cell attachment and migration. Attachment of endothelial cells to Del1 was associated with clustering of alphavbeta3, the formation of focal **complexes**, and recruitment of talin and vinculin into

these **complexes**. These events were shown to be associated with phosphorylation of proteins in the focal **complexes**, including the time-dependent phosphorylation of p125FAK, MAPK, and Shc. When recombinant Del1 was evaluated in an in ovo chick chorioallantoic membrane assay, it was found to have potent angiogenic activity. This angiogenic activity was inhibited by a monoclonal antibody directed against alphavbeta3, and an RAD mutant Del1 protein was inactive. Thus Del1 provides a unique autocrine angiogenic pathway for the embryonic endothelium, and this function is mediated in part by productive ligation of integrin alphavbeta3.

L3 ANSWER 14 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1999:34295 BIOSIS
DN PREV199900034295
TI Molecular interactions between the urokinase receptor and integrins in the vasculature.
AU May, A. E.; Kanse, S. M.; Chavakis, T.; Preissner, K. T. (1)
CS (1) Haemostasis Res. Unit, Kerckhoff-Klinik, Max-Planck-Institut, Sprudelhof 11, D-61231 Bad Nauheim Germany
SO Fibrinolysis & Proteolysis, (July, 1998) Vol. 12, No. 4, pp. 205-210. ISSN: 1369-0191.
DT General Review
LA English
AB Cell-cell and cell-ECM interactions are key events in morphogenic processes during developmental and reproductive phases, in immune defense, wound healing and tissue repair, or hemostasis. Their dysregulation plays a major role in the pathophysiology of cardiovascular diseases (atherosclerosis, restenosis, thrombosis) or **angiogenesis**-driven tumor progression. Protease cascades such as the plasminogen activation system are linked to cell adhesion and migration. The urokinase-type plasminogen activator (uPA) as well as its receptor (uPAR) has been found in a **complex** with beta1-, beta2-, and beta3-integrins, thereby allowing mutual interactions and regulatory processes between cell adhesion and proteolysis to occur. Moreover, both UPAR and PAI-1 are capable of binding to vitronectin, an adhesive extracellular matrix protein, that serves as ligand for vascular integrins in an **RGD** -dependent manner. This short review will focus on the molecular and functional interactions between the uPAR system and vascular integrins and discuss consequences for vascular cell functions.

L3 ANSWER 15 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1998:505448 BIOSIS
DN PREV199800505448
TI Accutin, a new disintegrin, inhibits **angiogenesis** in vitro and in vivo by acting as integrin alphavbeta3 antagonist and inducing apoptosis.
AU Yeh, Chia Hsin; Peng, Hui-Chin; Huang, Tur-Fu (1)
CS (1) Dep. Pharmacology, Coll. Med., Natl. Taiwan Univ., No. 1, Sec. 1, Jen-Ai Rd., Taipei Taiwan
SO Blood, (Nov. 1, 1998) Vol. 92, No. 9, pp. 3268-3276. ISSN: 0006-4971.
DT Article
LA English
AB Endothelial integrins play an essential role in **angiogenesis** and call survival. Accutin, a now member of disintegrin family derived from venom of Agkistrodon acutus, potently inhibited human platelet aggregation caused by various agonists (e.g., thrombin, collagen, and, adenosine diphosphate (ADP)) through the blockade of fibrinogen binding to platelet glycoprotein IIb/IIIa (i.e., integrin alphaIIbbeta3). In this report, we describe that accutin specifically Inhibited the binding of monoclonal antibody (MoAb) 7E3, which recognizes integrin alphavbeta3, to human umbilical vein endothelial cells (HUVECs), but not those of other anti-integrin MoAbs such as alpha2beta1, alpha3beta1, and alpha5beta1. Moreover, accutin, but not the control peptide GRGES, dose-dependently

inhibited the 7E3 interaction with HUVECs. Both 7E3 and GRGDS, but not GRGES or Integrelin, significantly blocked fluorescein isothiocyanate-**conjugated** accutin binding to HUVEC. In functional studies, accutin exhibited inhibitory effects on HUVEC adhesion to immobilized fibrinogen, fibronectin and vitronectin, and the capillary-like tube formation on Matrigel in a dose- and **RGD**-dependent manner. In addition, it exhibited an effective antiangiogenic effect in vivo when assayed by using the 10-day-old embryo chick CAM model. Furthermore, It potently induced HUVEC apoptotic DNA fragmentation as examined by electrophoretic and flow cytometric assays. In conclusion, accutin inhibits **angiogenesis** in vivo and in vitro by blocking integrin alphavbeta3 of endothelial cells and by inducing apoptosis. The antiangiogenic activity of disintegrins might be explored as the target of developing the potential antimetastatic agents.

L3 ANSWER 16 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1998:208076 BIOSIS
DN PREV199800208076
TI A role for tissue factor in cell adhesion and migration mediated by interaction with actin-binding protein 280.
AU Ott, Ilka; Fischer, Edgar G.; Miyagi, Yohei; Mueller, Barbara M.; Ruf, Wolfram (1)
CS (1) Dep. Immunol. Vascular Biol., IMM-17, Scripps Res. Inst., 10550 North Torrey Pines Road, La Jolla, CA 92037 USA
SO Journal of Cell Biology, (March 9, 1998) Vol. 140, No. 5, pp. 1241-1253. ISSN: 0021-9525.
DT Article
LA English
AB Tissue factor (TF), the protease receptor initiating the coagulation system, functions in vascular development, **angiogenesis**, and tumor cell metastasis by poorly defined molecular mechanisms. We demonstrate that immobilized ligands for TF specifically support cell adhesion, migration, spreading, and intracellular signaling, which are not inhibited by **RGD** peptides. Two-hybrid screening identified actin-binding protein 280 (ABP-280) as ligand for the TF cytoplasmic domain. Extracellular ligation of TF is necessary for ABP280 binding. ABP-280 recruitment to TF adhesion contacts is associated with reorganization of actin filaments, but cytoskeletal adaptor molecules typically found in integrin-mediated focal contacts are not associated with TF. **Chimeric** molecules of the TF cytoplasmic domain and an unrelated extracellular domain support cell spreading and migration, demonstrating that the extracellular domain of TF is not involved in the recruitment of accessory molecules that influence adhesive functions. Replacement of TF's cytoplasmic Ser residues with Asp to mimic phosphorylation enhances the interaction with ABP-280, whereas Ala mutations abolish coprecipitation of ABP-280 with immobilized TF cytoplasmic domain, and severely reduce cell spreading. The specific interaction of the TF cytoplasmic domain with ABP-280 provides a molecular pathway by which TF supports tumor cell metastasis and vascular remodeling.

L3 ANSWER 17 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1996:239487 BIOSIS
DN PREV199698787616
TI Alternative adhesion sites in human fibrinogen for vascular endothelial cells.
AU Thiagarajan, Perumal; Rippon, Amy J.; Farrell, David H. (1)
CS (1) Pa. State Univ., Coll. Med., P.O. Box 850, Hershey, PA 17033 USA
SO Biochemistry, (1996) Vol. 35, No. 13, pp. 4169-4175. ISSN: 0006-2960.
DT Article
LA English
AB Fibrinogen mediates endothelial cell adhesion, spreading, and **angiogenesis** through integrin alpha-v-beta-3. Previous studies by

several investigators have suggested that the Arg-Gly-Asp (**RGD**) site at position 572-574 on the alpha chain of human fibrinogen can bind to alpha-v-beta-3. However, this **RGD** sequence is absent in fibrinogen from most other species, including bovine, hamster, monkey, mouse, pig, and rat fibrinogen. In these species, an **RGD** site exists at the equivalent of position alpha-252-254, which has the sequence RGG in humans. In addition, the role of an integrin binding site on the gamma chain at position 400-411 has been an issue of controversy. In the present studies, recombinant fibrinogen molecules with mutations in the potential endothelial cell binding sites have been used to test the role of these sites directly. The results show that the **RGD** at alpha-572-574 is the primary adhesion site, and that the gamma chain site plays no significant role. Human and bovine plasma fibrinogens were also assayed for their ability to support adhesion of human and bovine vascular endothelial cells. The results show that although the two types of fibrinogen have **RGD** sequences at widely divergent sites, there is no significant difference in their ability to support endothelial cell adhesion. Furthermore, a **chimeric** human fibrinogen molecule with an **RGD** sequence at the bovine site, position alpha-252-254, also supported adhesion. These results indicate that an **RGD** site in human fibrinogen at either position alpha-252-254 or position alpha-572-574 can mediate endothelial cell adhesion.

L3 ANSWER 18 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1996:188524 BIOSIS
DN PREV199698744653
TI Human microvascular endothelial cells adhere to thrombospondin-1 via an **RGD**/CSVTCG domain independent mechanism.
AU Chen, Zhong-Sheng; Pohl, Jan; Lawley, Thomas J.; Swerlick, Robert A. (1)
CS (1) Dep. Dermatol., Emory Univ. Sch. Med., WMB 5014, Atlanta, GA 30322 USA
SO Journal of Investigative Dermatology, (1996) Vol. 106, No. 2, pp. 215-220. ISSN: 0022-202X.
DT Article
LA English
AB Thrombospondin-1 (TSP-1), a 450-kDa glycoprotein secreted by platelets and endothelial cells at sites of tissue injury or inflammation, plays an important role in **angiogenesis**, inflammation, and vascular occlusive skin diseases. Many of the physiologic and pathologic activities of TSP-1 are dependent upon its interactions with endothelial cells. To better understand the basis of these activities, we examined the mechanisms mediating the binding of human dermal microvascular endothelial cells (HDMEC) to immobilized TSP-1. HDMEC bound to but did not spread on TSP-1 in a concentration-dependent manner. Monoclonal antibodies (MoAbs) which recognize two purported TSP-1 binding proteins, CD36 and the alpha-v integrin chain, or TSP-1-derived peptides CGRGDS and CSVTCG, alone or in combination with heparin, did not inhibit HDMEC adhesion to immobilized TSP-1. Furthermore, CSVTCG-ovalbumin **conjugates** failed to support HDMEC adhesion. Although **RGD**-containing peptides immobilized on plastic wells supported HDMEC binding, they also induced cell spreading not characteristic of cell binding to TSP-1 and binding was inhibited by free **RGD** peptide. Two MoAbs against different domains of TSP-1 (A4.1 and C6.1) failed to block HDMEC binding to TSP-1, but both MoAbs inhibited G361 human melanoma cell binding to TSP-1 by 60%. Acid treatment of TSP-1 almost completely abrogated its ability to support HDMEC binding, while acid treatment inhibited G361 binding by 50%. However, either antibody completely abrogated G-361 cell binding to acid-treated TSP-1. These data demonstrate that HDMEC bind to immobilized TSP-1 in an **RGD**- and CSVTCG-independent manner via an acid labile epitope(s) which is recognized via a receptor or receptors distinct from CD36 or alpha-v-beta-3 integrin receptor.

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     FILE 'BIOSIS' ENTERED AT 17:22:51 ON 18 JUL 2003
L1         23483 S ANGIOGEN?
L2        609823 S CONJUGAT? OR CHIMERIC OR COMPLEX?
L3          1230 S L1 AND L2
L4           118 S RGD AND ANGIOGEN?
L5            23 S L2 AND L4
L6           896 S ANGIOSTATIN OR ENDOSTATIN
L7          2963 S RGD
L8             6 S L6 AND L7
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